

Aus dem Gerinnungs- und Fibrinolyselabor
(Leitung: Dr. F. Duckert) der Medizinischen Universitätsklinik
(Prof. F. Koller) des Bürgerspitals Basel

**The Monoiodoacetate (MIA) Tolerance
Test, a New Quantitative Method for the
Fibrin Stabilizing Factor (Factor XIII)
Assay**

INAUGURAL-DISSERTATION

zur Erlangung der Doktorwürde der medizinischen Fakultät
der Universität Basel

vorgelegt von

PETER SIGG

Dörflingen und Schaffhausen



F. K. SCHATTAUER-VERLAG · STUTTGART 1965



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Von der medizinischen Fakultät Basel genehmigt
auf Antrag von Professor F. KOLLER

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CURRICULUM VITAE

Ich wurde am 6. 10. 34 in Winterthur geboren. Die Primarschule absolvierte ich in Kloten, das Realgymnasium und das Medizinstudium, mit Ausnahme von je einem Semester in Rom und in Paris, in Zürich. Das Staatsexamen bestand ich im Herbst 1960, anschließend war ich in Wien als Volontärassistent an der I. Universitätsfrauenklinik (Prof. T. ANTOINE) tätig. Die Jahre 1962 und 1963 arbeitete ich im Gerinnungsphysiologischen Labor zuerst in Zürich, dann in Basel unter der Leitung von Dr. DUCKERT und Prof. F. KOLLER. Seit dem 1. 1. 64 bin ich Assistent am Kinderspital Zürich (Prof. A. PRADER).

Introduction

The fibrin stabilizing factor (FSF, factor XIII) was first described by Robbins (18) in 1944 and more precisely characterized by Laki and Lorand (12) in 1948. In its absence a fibrin clot is soluble in 5M urea. A congenital lack of FSF has been described in two sibling who were suffering from severe haemorrhages and poor wound healing (4, 19). While the congenital total lack of FSF has been reported by several investigators (8, 9, 15, 16), the acquired form in such degree as to cause bleeding has not been described.

To assay FSF several tests are known but they are all indirect methods. They are mostly based on testing plasma in various dilutions on an FSF free fibrinogen. In the low range of FSF activity the plasma to be tested is used undiluted and its own fibrinogen interferes with the determination. Moreover to obtain qualitatively constant fibrinogen devoid of FSF is not easy, for the separation of fibrinogen from FSF is a time consuming procedure and alterations are likely to happen. Pure fibrinogen may be obtained from patients with FSF deficiency but this condition is extremely rare.

We aimed therefore to elaborate a simple method for the FSF assay without using FSF free fibrinogen.

It is well known that:

1. Two factors, i.e. calcium and FSF, are necessary for the conversion of urea soluble fibrin (fibrin s) to the physiological urea insoluble fibrin (fibrin i).
2. Monoiodoacetic acid (MIA) and other related agents acting on sulphydryl groups can inhibit fibrin i formation even in presence of calcium and FSF.

Based on these facts we devised the MIA tolerance test. It is possible to demonstrate that the effect of the MIA on the fibrin stability in 5 M urea depends

directly on the concentration of factor XIII, and to a certain extent on the relationship of factor XIII to fibrinogen, when the latter reaches extremely high or low values.

Reagents

Veronal-acetate buffer pH 7.35 (Michaelis)

a) Stock solution: CH_3COONa . 3 H_2O , 9.714 g. Na-Veronal, 14.714 g. Dissolve the mixture and bring the total volume to 500 ml with distilled water.

b) Buffer solution: Stock solution 250 ml, 4.25% NaCl 200 ml, N/10 HCl 217 ml, distilled water 683 ml.

BaSO₄: prepared for x ray examination by Merck, Germany.

Thrombin: bovine thrombin "Roche" 55 N.I.H. U/mg protein dissolved in veronal-acetate buffer to have a concentration of 100 N.I.H. U/ml kept at -25°C in silicone coated tubes.

Iodoacetic acid sodium salt: ICH_2COONa (= MIA). Solution in distilled water.

Ammonium sulphate: saturated at room temperature. The pH is adjusted to 7.0 with concentrated NH_4OH .

Urea: 5 M solution.

Buffered saline: 9 parts saline, 1 part veronal-acetate buffer.

Heparin: "Vitrum" in 5% solution (5000 U/ml).

Methods

Clot solubility test: According to Loewy and Edsall (13) and Lüscher (14). The test consists in incubating either plasma or fibrinogen clots in 3 ml of 5 M urea at 37°C . The clotting system is variable and will be indicated for each determination. In all cases the clots are incubated at 37°C for 30 min before the addition of the urea solution. The clots have to be loosened from the tubes and must swim in the solution.

The test is used for the control of the absence of either the fibrin stabilizing factor or calcium.

Prothrombin assay: Koller, Loeliger and Duckert (11).

Factor V assay: Kappeler (10).

Factor VII assay: Zollinger (22).

Factor IX assay: Geiger, Duckert and Koller (7).

Factor X assay: Bachmann, Duckert and Koller (1).

Fibrinogen determination: Clauss (3).

Antithrombin determination: Duckert (5).

Calcium poor plasma: Plasma taken from oxalated blood is dialysed against veronal-acetate buffer for 18 hours.

All the following operations are carried out at $2-4^\circ\text{C}$ if not otherwise stated.

1. FSF Rich Fibrinogen

Human oxalated blood (9 parts venous blood and 1 part sodium oxalate M/10) is collected in silicone-coated tubes and the plasma separated by centrifugation. 100 ml of plasma are treated with BaSO_4 at room temperature for 10 min.

The BaSO_4 -adsorbed plasma is centrifuged for 10 min at 1500 g. The supernatant is salted out with saturated ammonium sulphate solution pH 7.0 at a final concentration of 27%. It is then

centrifuged at 1500 g for 20 min and the sediment is dissolved in cold saline. The salting out procedure is then repeated at 25% final concentration. The sediment is again dissolved in a few ml of distilled water and minimal amounts of saline. The solution is dialysed against buffered saline until complete disappearance of sulphate ions. FSF rich fibrinogen solution can be kept at -25°C for several months without loss of activity.

2. FSF Free Fibrinogen

FSF rich fibrinogen solution is incubated for 3–5 hours at 40°C . This method gives very variable results: it is not always possible to prevent fibrinogen denaturation and to attain complete destruction of FSF.

3. FSF Free Thrombin

This FSF free thrombin is obtained by converting prothrombin into thrombin by means of the intrinsic activator.

a) FSF Free Prothrombin

Oxalated human plasma is precipitated with ammonium sulphate at 34% saturation, left for 10 min at 4°C before centrifugation at 1500 g for 20 min. Fibrinogen and FSF are in the sediment. The supernatant is dialysed against buffered saline as long as SO_4^{--} ions are detected.

The dialysed solution is oxalated by 1/9 of its volume sodium oxalate M/10 and treated with BaSO_4 during 10 min (5 g/100 ml). The suspension is centrifuged for 5 min at 1500 g. After removal of the supernatant the BaSO_4 is thoroughly washed with saline several times to eliminate protein impurities. After these washings the BaSO_4 is treated with sodium citrate 0.14 M at pH 7.0 for 30 min. The amount of eluting fluid is 1/5 of the volume of the starting plasma. After centrifugation for 10 min it is dialysed against distilled water for 48 hours at 4°C . The prothrombin solution contains 50–70 U prothrombin/ml. It is contaminated with factor VII, factor IX and factor X which do not disturb the experiments.

b) Isolation of Intrinsic Activator

For the intrinsic activator preparation a system similar to the modified thromboplastin generation test by Duckert et al. (6) has been used. Instead of 0.3 ml we incubated 1.0 ml of each of the four constituents, i.e. adsorbed plasma, serum, lipids and calcium chloride. When the highest intrinsic activator activity is reached as tested on Dowex decalcified plasma, the incubation mixture is cooled down to 0°C .

The fibrin traces are removed by gentle stirring of the solution with a clean wooden rod.

The suspension is then centrifuged at 25 000 g at 0°C for 30 min. The supernatant is discarded and the sedimented intrinsic activator is resuspended in a mixture of CaCl_2 and veronal-acetate buffer as follows:

0.5 ml veronal-acetate buffer

0.5 ml CaCl_2 1/80 M

The final CaCl_2 concentration is the same as in the formation system for intrinsic activator activity.

Immediately after suspension the intrinsic activator is used for the prothrombin conversion. The intrinsic activator is tested with FSF free plasma for absence of FSF (see below).

c) Prothrombin Conversion

FSF free prothrombin is incubated at 37°C with intrinsic activator until maximal thrombin activity can be demonstrated.

- 1.0 ml FSF free prothrombin
- 1.0 ml FSF free intrinsic activator

The thrombin activity is tested on a 0.2% fibrinogen solution. After ca. 15 min incubation, the thrombin reaches its highest degree of activity. The solution is put into siliconised tubes and kept deep frozen at -25°C .

This thrombin solution is FSF free.

d) Test for FSF Absence

All products of human origin, prothrombin, intrinsic activator and thrombin as well as bovine thrombin are tested for FSF absence in the following system:

- 0.2 ml FSF deficient plasma or FSF free fibrinogen
- 0.1 ml CaCl_2 M/20
- 0.1 ml prothrombin, or intrinsic activator, or thrombin

After incubation at 37°C for 30 min 3 ml of 5 M urea are added. The system is further incubated at 37°C . In the case of FSF absence the clot will be dissolved in less than 2 hours, rarely 4 hours, otherwise there will be no signs of clot dissolution in 24 hours.

Prothrombin, intrinsic activator and thrombin are free of FSF. Table 1 gives the results for the thrombin reagent.

Table 1. Absence of FSF in Thrombin Reagents, Tested with FSF Free Plasma.

	Clot dissolution time in 5 M urea
Human thrombin	26 mins.
Bovine thrombin	25 mins.
Buffer	24–26 mins.

4. Calcium Poor Thrombin

Thrombin "Roche" is dissolved in veronal-acetate buffer to attain a final concentration of 100 N.I.H. U. thrombin/ml. This solution is dialysed against veronal-acetate buffer for 18 hours at $2-4^{\circ}\text{C}$ and then deep frozen and kept in silicone coated tubes at -25°C .

Test for the absence of calcium:

- 0.2 ml FSF rich fibrinogen are clotted with
- 0.1 ml thrombin solution (33 N.I.H. U./ml)

The clot solubility depends on the calcium concentration of the test system. The clots readily dissolved in 25 min. When 0.1 ml CaCl_2 is added in various dilutions to 0.2 ml calcium poor plasma clotted with 0.1 calcium poor thrombin we found the clots to be urea soluble below a CaCl_2 concentration of M/400.

Performance of the MIA Tolerance Test

We prefer oxalated blood to citrated blood: recalcified oxalated plasma gives a slightly opaque clot which can be more easily distinguished in the urea solution than the almost transparent citrated plasma clot.

The following clotting mixtures:

- 0.2 ml plasma to be tested

0.1 ml MIA in the standard concentrations: 0.035, 0.025, 0.015 and 0.005 M
 0.1 ml CaCl_2 M/20
 are incubated for 30 min at 37° C in glass tubes (diameter 12.5 mm). 3 ml of urea 5 M are then added and the timer is started. Immediately after the addition of urea the clots are gently removed from the glass wall. They must not be damaged at all and should swim in the suspending medium. The tubes are not touched except at the control times i.e. after 30, 45, 60, and 90 min when they are vigorously shaken.

The progressing of the clot dissolution in urea is recorded according to the following criteria and the corresponding value (4-0) is attributed to each clot. This is illustrated by Fig. 1.

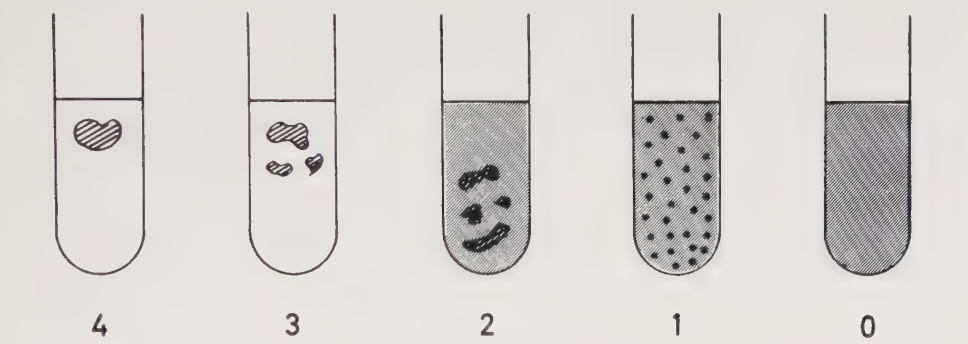


Fig. 1. *Criteria and valuation of the MIA tolerance.* (By the kind permission of the Benno Schwabe Verlag, Basel.) 4: Intact clot swimming in clear solution. 3: Slightly decomposed clot swimming in clear urea solution. 2: Heavily decomposed clot swimming in turbid solution, the clot is split in several pieces. 1: Opaque solution with small, nearly no more visible particles. 0: Homogenous, opaque solution without any particles. (21)

These values are recorded as indicated in Fig. 2.

MIA concentration				
0.035 M	2	2	1	0
0.025 M	3	3	2	1
0.015 M	4	4	4	4
0.005 M	4	4	4	4
Incubation time	30	45	60	90 mins.

Example of MIA Tolerance.

Fig. 2. In this example the MIA tolerance is 46.

Definition of the MIA Tolerance

The sum of the sixteen numbers listed in the square shown in Fig. 2 gives the values of the MIA tolerance. It is proportional to the FSF concentration as it

will be demonstrated by means of the calibration curve. When MIA is added in various concentrations to plasma which is then clotted, incubated and suspended in urea, the dissolution time of those clots depends on the MIA and FSF concentration.

The Calibration Curve

The calibration curve is obtained by mixing a normal plasma having a theoretical FSF concentration of 100% with totally FSF deficient plasma. The two plasmas should have a similar fibrinogen concentration. The calibration curve is given in Fig. 3.

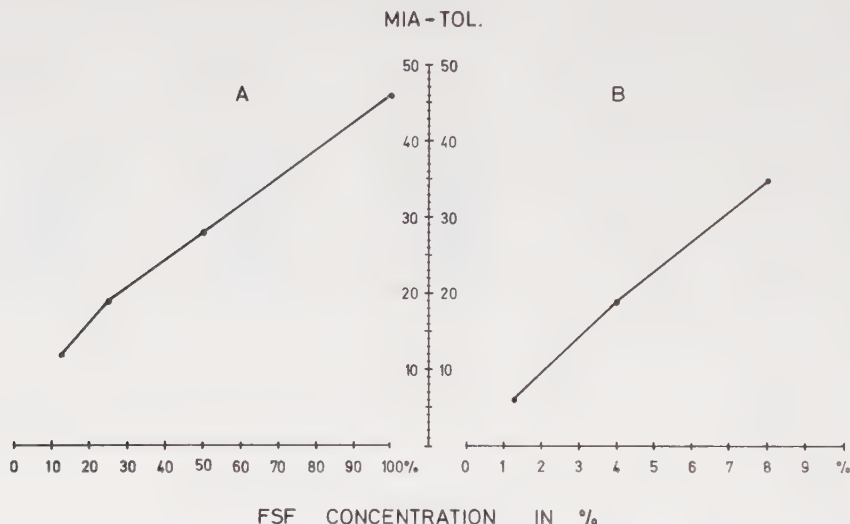


Fig. 3. *MIA tolerance, calibration curve.* The following MIA concentrations were used for the determination of the calibration curves. A: MIA concentrations of 0.035, 0.025, 0.015, and 0.005 (Standard) M. B: 0.005, 0.002, 0.001, and 0.0005 M. For FSF concentrations below 10%.

The calibration curve shows the MIA tolerance in function of the FSF concentration. The optimal range (Fig. 3 curve A) is between 50% and 12%. Below 12% FSF activity the test is no more sensitive to variations of the FSF concentration.

By further dilution of the standard MIA solutions (Fig. 3 curve B) we were able to determine highly decreased clot resistance. The clots become more resistant against urea. The method is therefore made quantitative for lower ranges of FSF activity. For this purpose we used MIA concentrations of 0.005, 0.002, 0.001, and 0.0005 M. These two calibration curves enabled us to determine quantitatively FSF concentrations of various degrees.

Reproducibility of the MIA Tolerance under Various Conditions

The MIA tolerance was determined in plasmas collected on various anti-coagulants. 9 ml blood taken by venipuncture were added to 1 ml anticoagulant solutions. These were prepared as follows:

1. Sodium oxalate M/10
2. Sodium citrate M/10
3. 20 I. U. Heparin "Vitrum"/ml veronal-acetate buffer.

All these plasma samples were kept under different conditions, i.e. at -25°C , $+4^{\circ}\text{C}$, and at room temperature.

The MIA tolerance is not altered in two days under any of the above mentioned conditions. Longer incubation alters the coagulability of the non frozen samples. In citrated blood the MIA tolerance is less changed: stored for 24 days at 4°C the plasma is still coagulable and the MIA tolerance only slightly diminished. The MIA tolerance of the deep frozen plasmas is quite constant, after 40 days slightly reduced in oxalated plasmas. This might be attributed to alterations of either the fibrinogen or the FSF molecule. Further studies on this subject have to be done.

The practical consequence of this investigation is, that the MIA test need not be performed immediately after venipuncture. Plasma can be kept at 4°C several days before the performance of the test will be done. It is however advisable to determine the MIA tolerance soon after the collection of the blood in order to avoid any cause of uncertainty.

The Normal Range of the MIA Tolerance

10 healthy blood donors had an average MIA tolerance of 45.5. This value was taken for 100% in the calibration curve. We have no explanation for the fact that in patients have a lower MIA tolerance. 42 in patients had an average MIA tolerance of 32. Of these 42 patients 22 had an average MIA tolerance of 35 and also normal clotting factors (Quick time over 60%, Antithrombin between 13–18 secs. Fibrinogen between 200 and 500 mg%).

10 successive determinations in the same plasma of a normal donor give only small variations as it can be seen in Table 2.

Table 2. Successive Determination in the Same Plasma of a Normal Blood Donor.

MIA tolerance	46	45	44	46	45	48	46	45	45	46
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The MIA Tolerance in Function of the pH of the Urea Solution

The pH of 5 M urea solution was adjusted with H_2SO_4 and NH_4OH respectively to 5.2 or 8.0. The influence of these various urea solutions on the MIA

tolerance has been tested on plasmas at 100% and 20% FSF level respectively. Although little variations can be seen, there is no constant influence of the pH on the MIA tolerance. Under usual conditions the urea solution will never attain such extreme values of the pH as 5.2 or 8.0 respectively. Therefore the MIA tolerance test can be performed without control of the pH.

Reversibility of the MIA Action on Fibrin Stability

Normal plasma is mixed with a MIA solution, concentrated enough in order to block entirely the formation of fibrin i, without complete inhibition of the coagulation process.

This MIA treated plasma was then dialysed in an open system at 4° C against buffered saline. The latter was frequently renewed. At different time intervals aliquots are removed and tested for clot solubility after recalcification and 30 mins. incubation at 37° C. If a clot did not dissolve in 5 M urea in 30 mins., an MIA tolerance test was performed with plasma taken at the same time out of the dialysing system. The results are given in the Table 3.

After a dialysing time of two hours the clot dissolved in 30 mins. The MIA was therefore fully effective. After 18 hours the clot remained insoluble, the MIA tolerance was normalized and returned to its original value. The effect of inhibiting FSF due to preincubation with MIA is totally reversible, when the iodoacetate is eliminated by simple dialysis.

Table 3. The MIA Tolerance in Relation to the Dialysing Time of MIA Treated Plasma.

Dialysing time in hours	2	18	21	27	41	49
Clot dissolution time in mins. below 30'	over 2 hours					
MIA tolerance	0	35	32	34	34	20

Effect of Variation of the Incubation Time before Addition of 5 M Urea in the Standard MIA Test System

In Loewy's clot resistance test (13) the recalcified plasma clot is incubated for 30 mins. at 37° C before the addition of urea. During this time the fibrin should be stabilized. MIA addition might alter the situation. In the following experiment we tested the influence of the incubation time on the MIA tolerance at various FSF levels.

1. Plasma with 100% FSF from a normal blood donor.
2. Plasma with 33% FSF prepared by dilution of the normal plasma with an FSF deficient plasma of the same fibrinogen content.
3. Plasma mixed in a similar way to reach on FSF concentration of 10% of the normal activity.

With those three plasmas we performed the MIA tolerance test varying the incubation time before adding urea to the clots. The values at 30 mins. incubation time correspond nearly to the values of the calibration curve. The results are given in Table 4.

Table 4. The MIA Tolerance in Relation to the Incubation Time of Plasma at Various FSF Concentrations.

Theoretical FSF concentr. in mixed plasmas	Incubation time after clotting									
	5''	15''	75''	3'	4'	5'	7'	10'	20'	30'
100% FSF	53			55	55	55			53	48
33% FSF		11	22	23		22	25			19
10% FSF		0 ¹⁾	0 ¹⁾	5		6		8		11

It shows the MIA tolerance in relation to the incubation time after recalcifying the plasma and before the urea addition. The interpretation of the results from very short incubation times is difficult for the clotting time of each tube is slightly different from the other and hence the clotting moment can not be predicted accurately.

At concentrations between 100% and 33% FSF seems to be very effective immediately after the coagulation. At 10% FSF the reaction rate is strongly diminished and the stabilizing effect is not immediate.

Prolonged Incubation Time of the Recalcified Clot before 5 M Urea Addition

In the original test system the clots are incubated 30 mins. before addition of urea. If this incubation time is prolonged, a slight reduction of the MIA tolerance results. The decrease is relatively more accentuated in plasma with reduced FSF concentration. The results are given in Table 5.

Table 5. The MIA Tolerance in Relation to the Incubation Time of MIA Treated Clots after Coagulation at Various FSF Concentrations.

Theoretical FSF concentr.	Incubation time after clotting			
	30'	60'	120'	180'
100% FSF	35	35	30	29
33%	18	18	14	15

The MIA tolerance test performed with higher MIA concentrations (0.05, 0.04, 0.03, and 0.02 M) and prolonged incubation time lead to similar results, which are given in Table 6.

1) A solid clot is formed, but it is not resistant to urea.

Table 6. The MIA Tolerance of Normal Plasmas with Different MIA Dilutions than those Used in the Standard Test System: 0.05, 0.04, 0.03, and 0.02 M in Relation to the Incubation Time after Coagulation.

	Incubation time after clotting			
	30'	60'	120'	180'
Plasma with 100% FSF	12	12	8	8

Again there is a demonstrable tendency towards decreasing MIA tolerance after a longer incubation period.

Discussion

The known methods for the FSF assay are based on the following principle: Plasma is coagulated and the clot is suspended in urea or monochloroacetic acid. The dissolution time is recorded. A time of about 30 mins. corresponds to a total lack of FSF. Such a plasma can be taken as a substrate for the quantitative assay of factor XIII. One determines the highest dilution of the substance to be tested which added to the substrate gives an insoluble clot. This critical dilution is proportional to the FSF concentration (4). FSF free plasma can be replaced by FSF free fibrinogen (12, 13).

These methods are not without difficulties, FSF free plasma is difficult to obtain. Until now only few patients are known with a total lack of FSF (4, 8, 9, 15, 16).

One must keep in mind, that traces of FSF are sufficient for the fibrin i formation. Contamination of the test system by FSF, e.g. by FSF containing thrombin, may easily occur. FSF and fibrinogen are difficult to separate because of physical and chemical similarities. The isolation procedures may alter their molecular structure and cause denaturation with loss of activity.

The advantage of the MIA tolerance test is, that the FSF concentration can be measured directly without FSF free substrate and without manipulation of the plasma. The MIA tolerance is directly proportional to the FSF concentration. However the FSF activity is dependent on the fibrinogen-FSF relation when the fibrinogen reaches extreme values. The MIA tolerance test is therefore suitable for clinical use to test material containing fibrinogen. FSF free substrate is not necessary except for the calibration curve. We could demonstrate in our dialysing experiment, that the inhibition of FSF by MIA is reversible. It looks like a competition between MIA and FSF, because the clot solubility depends on the relation of FSF to MIA. At higher concentrations MIA makes the plasma behave like an FSF deficient one.

We may explain the action of MIA on the stability of the clot with the following hypotheses. Swigert et al. showed 1963 (20) that fibrin is urea soluble when fibrinogen is previously incubated with para-chloromercuribenzoate. This reaction is irreversible. FSF is denaturated and fibrinogen is unaltered because after addition of intact enzyme (FSF) it can be converted into insoluble fibrin.

With MIA we are working under other conditions. It is possible that FSF and MIA are in an equilibrium with an inactive FSF-MIA complex and FSF is reversibly inhibited. When the MIA concentration is high enough, we have a total inhibition, which still is reversible. According to the other hypothesis FSF and MIA would compete for the same site on the fibrin molecule. The fibrin aggregation, i.e. the formation of the fibrin molecule is not influenced by MIA at the concentrations used for the MIA tolerance test. The appearance of the fibrin network is not delayed.

The presence of MIA reduces the apparent activity of FSF to the critical level. This reduction is naturally proportional to the MIA concentration in the system. At low FSF levels the equilibrium between FSF and MIA is attained at correspondingly lower MIA concentrations. This mechanism makes the system suitable for the quantitative determination.

As we could demonstrate, above 10% the FSF action is very fast. Immediately after the coagulation the fibrin is fully stabilized. Only at relatively low concentrations, i.e. below 10% this reaction goes on slowly in presence of MIA. This situation might be compared with that of an FSF deficient plasma. The traces of active FSF left in this blood might react very slowly and might never suffice for the formation of a stable fibrin network.

Calcium is absolutely necessary for the reaction between FSF and fibrin. But already small amounts of calcium are sufficient to produce an insoluble fibrin clot. We showed, that a CaCl_2 concentration of 1/400 M, which is twenty times lower than that of the standard CaCl_2 concentration in the MIA tolerance test is still enough for full fibrin stability.

The other coagulation steps need calcium in higher concentration. Hypocalcemia can never be the limiting factor in the fibrin-stabilizing reaction. It can therefore never be the cause of haemorrhages due to the lack of fibrin formation.

The MIA tolerance decreases when the FSF concentration is low or the fibrinogen concentration high. This is due to the fact, that the FSF action is dependent on the quantitative relation of FSF to fibrinogen. We have to distinguish between apparent and true FSF deficiency and may illustrate this with two clinical situations.

1. Hyperfibrinogenaemia due to e.g. infectious disease showed a marked reduction of the MIA tolerance.

2. FSF deficiency could be observed either acquired in conditions of liver cirrhosis and lung cancer (17) or inherited in heterozygote members of the family with FSF deficiency.

The MIA tolerance test should therefore always be performed together with the fibrinogen determination.

We never observed such severe FSF decrease, true or apparent, that would provoke direct urea solubility. No isolated acquired FSF deficiency is known to produce a bleeding tendency. If the clotting factors are reduced close to the limit of inducing haemorrhages, a coexistent low FSF concentration is unlikely to cause bleeding. We could not observe in our patients with congenital FSF deficiency spontaneous haemorrhages, they occurred only after a short period (24–36 hours) of perfect haemostasis following the trauma. A soluble fibrin network is only sufficient for stopping the bleeding temporarily. Proteolytic enzymes might digest such a clot before the fibroblasts are able to organize the clot. In addition it is known (2), that the growth of the fibroblasts is delayed and insufficient in fibrin clots.

The substrate fibrin is also dependent on the amount of fibrinogen present in the plasma. The speed of fibrin formation however is dependent on fibrinogen and thrombin. Experiments with FSF free human thrombin given to FSF rich plasma demonstrate that though the MIA tolerance is slightly altered in relation to the thrombin concentration, thrombin is not a limiting factor for the fibrin stability. The results are not easy to interpret and must be subject to further studies.

The thrombin formation time will not interfere in most of the clinical cases, except when the clotting time of plasma is near 30 mins. Therefore we recommend the addition of small amounts of FSF free human or bovine thrombin to the calcium solutions used for the MIA tolerance test. The clot formed is then not dependent on the endogenous thrombin production and the stability is a function merely of FSF or fibrinogen. If factor XIII free thrombin is not available the incubation time can be slightly prolonged.

The MIA tolerance test is a good method for clinical use. Its performance is very easy and the reagents are cheap. A pathological MIA tolerance was not observed to produce haemorrhages, except when decreased to 0.

Summary

A new quantitative test for the fibrin stabilizing factor (FSF, factor XIII) determination is described. The test is based on the principle of the reversible inhibition of the FSF action by monoiodoacetate (MIA).

The definition of the MIA tolerance is given. The normal range is about 45. The MIA tolerance is studied under various conditions. No FSF free material is necessary for its performance.

Résumé

On décrit un nouveau test pour le dosage quantitatif du facteur stabilisant la fibrine (FSF, facteur XIII). La méthode est basée sur l'inhibition réversible de l'action du FSF par le monoiodoacétate de sodium (MIA).

On donne la définition de la tolérance au monoiodoacétate. La valeur normale est d'environ 45. La tolérance au MIA est étudiée sous diverses conditions. Le test ne requiert pas de matériel purifié dépourvu pas de facteur XIII.

Zusammenfassung

Es wird eine neue quantitative Methode für die Bestimmung des Fibrin-stabilisierenden Faktors (FSF, Faktor XIII) beschrieben. Der Test basiert auf dem Prinzip der reversiblen Hemmung der FSF-Wirkung durch Monojodazetat (MIA).

Es wird die Definition der Monojodazetattoleranz gegeben. Der Normalbereich ist ungefähr 45. Die MIA-Toleranz wird unter verschiedenen Bedingungen untersucht. Kein FSF-freies Material ist für die Durchführung des Testes erforderlich.

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